

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

ANSI*net*

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Status of Lipid Peroxidation and Antioxidant Enzymes in the Tissues of Rats Fed Low – Protein Diet

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Abstract: The effect of low-protein diet on the activity of antioxidant enzymes (Catalase and Superoxide dismutase) and the status of lipid peroxidation as assessed by malondialdehyde levels in the brain, liver kidney, lungs and heart of rats were studied. Male weanling rats were maintained on low protein diets (2% of protein in diet instead of 25%) for a period of four weeks. Malondialdehyde contents (levels) of tissues of animals fed low - protein diet was significantly increased ($P < 0.05$) when compared with the control. The heart recorded the highest level of malondialdehyde when compared with other tissues. The activities of superoxide dismutase and catalase were significantly increased in the brain and liver of rats fed low protein diet while a significant reduction was observed in the kidney and lungs. It may therefore mean that the ingestion of low- protein diet might led to increased tissue lipid peroxidation (oxidative stress) and altered the activity of antioxidant enzymes.

Key words: Lipid peroxidation, protein-energy malnutrition, catalase and superoxide dismutase

Introduction

Protein energy malnutrition (PEM) previously termed protein calorie malnutrition has assumed the position of being the leading cause of death directly or indirectly among children under 5 years of age in developing world in the past forty years (Ibukun Olu Alade, 2001), in spite of our wide knowledge and understanding of human nutritional requirements.

The term protein energy malnutrition applies to a group of related disorders that include marasmus, kwashiorkor, and intermediate states of marasmus-kwashiorkor. Marasmus involves inadequate intake of protein and calories and is characterized by emaciation. The term kwashiorkor refers to the disease that occur when there is inadequate protein intake with reasonable calorie (energy) intake (Ibukun Olu-Alade, 2001). Studies suggest that marasmus represents an adaptive response to starvation whereas kwashiorkor represents a maladaptive response to starvation (Berkow and Robert, 1999). Children may present with a mixed picture of marasmus and kwashiorkor or with milder form of malnutrition, for this reason, the term protein energy malnutrition was suggested to include both entities (Berkow and Robert, 1999).

Although the clinical features of protein energy malnutrition are well known, its pathophysiology is still unclear. The clinical manifestations of malnutrition may be evident on physical examination but biochemical alteration may not be visible initially (Talli *et al.*, 2000). Most reported studies were carried out using the Red blood cell as a model (Sive *et al.*, 1993; Ashour *et al.*, 1999, Talli *et al.*, 2000)

Free radicals are highly unstable molecules that interact

quickly and aggressively with the molecules in the body causing damage. They have been implicated in many diseases such as cancer, diabetes, hypertension, etc (Tisan *et al.*, 1995). The rise in free radicals, associated with antioxidant deficiency is said to result in tissue damage.

The pathogenesis of oedema and anaemia commonly found in children with protein energy malnutrition has been suggested to be caused by an imbalance between the production of these toxic radical and their safe disposal (Ashour *et al.*, 1999). The aim of this study therefore is to evaluate oxidant / antioxidant status in the tissues of rat fed low protein diet.

Materials and Methods

Animals and diet: Ten weanling male albino rat (*Rattus norvegicus*) obtained from the animal breeding unit of the Department of Biochemistry, University of Ilorin were divided into two groups consisting of five rats each and were maintained on the following diets:

- (i) Control diet containing adequate protein (25%) and served us control group.
- (ii) Low-protein diet containing 2% protein (Adelusi and Olowokere, 1985). which served as Test group. The composition of the test diet is shown in Table 1. Rats were fed their respective diets and water *ad libitum* for a period of 28 days.

Tissue preparations: At the end of the period, the two group of rats were sacrificed by anaesthetizing in a jar containing cotton wool soaked in chloroform. The tissues of interest (brain, liver, kidney and lungs) were removed, kidneys were decapsulated, washed free of

blood, weighed and immersed in -ice-cold 0.25M sucrose solution.

Tissues were homogenized separately in-ice-cold 0.25M sucrose buffer solution (x6 dilution). The homogenates were kept frozen overnight before enzyme assay to allow unbroken cells to lyse (Akanji, 1986).

Table 1: Diet composition (g/100g)

| Constituent | Quantity (g) |
|------------------|--------------|
| Soymeal | 40 |
| *Vitamin/min mix | 60 |
| Corn starch | 820 |
| Oil | 80 |
| | 1000 |

*Mineral mix contained (g/kg diet): CaCO₃ (15.258); CoCl₂.6H₂O(0.001); ZnCl₂ (0.001); CuSO₄.5H₂O (0.019); FeSO₄.7H₂O (1.078); MgSO₄ (2.929); MnSO₄.2H₂O (0.178); KI (0.032); KH₂PO₄ (15.559) and NaCl (5.573).
The vitamin mix contained (g/kg diet): Thiamine (0.02); Riboflavin (0.03); Pyridoxine (0.01); p-Aminobenzoic acid (0.20); Myo-inositol (2.00); Biotin (0.001); Menadione (0.01); Ergocalciferol (0.4); Choline-HCl (2.0) and Cellulose (3.31).

Soyabean meal contained 49.0% protein, 6.5% fibre and 31.8% carbohydrate. It was treated with EDTA (10g/l) (Kratzer *et al.*, 1959) and washed several times thereafter, dried and used for diet composition. Control feed contained 25% protein.

Determination of tissue peroxidation: The extent of lipid peroxidation in tissues was assessed by measuring the level of malondialdehyde in the various tissues (Wills, 1985).

Enzyme and protein measurement

Catalase: Catalase activity was determined in tissue homogenate according to the method of Ashru and Sinha (1971). In this method dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H₂O₂, perchromic acid is formed which is an unstable intermediate. The chromic acid produced is measured colorimetrically at 570nm.

Superoxide dismutase: The activity of superoxide dismutase in the various tissues was determined by the method of Misra and Fridovich (1972). The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase.

O₂ generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced by O₂ increases with increasing pH and also increases with increasing concentration of epinephrine.

Protein Determination: Protein concentration was

measured by the biuret method (Plummer, 1974). In alkaline medium, cupric ions form a purple coloured complex with compounds containing repeated CONH-links such as protein. The intensity of the colour is a measure of the number of peptide bonds in solution and hence the protein concentration.

All measurements were done using Spectronic 20 spectrophotometer. Student's T-test was carried out to determine the statistical significance of results.

Results

Compared with the control, malondialdehyde levels were found to be significantly (P<0.05) increased in all the tissues (brain, liver, kidney and lungs) of animals fed low-protein diet (Table 2). The brain recorded the highest levels of malondialdehyde when compared with other tissues. The levels of malondialdehyde were also found to be higher in the tissues of animals maintained on the diet containing low protein diet.

Catalase activity of the various rat tissues is shown in Table 3. The activity of catalase in the brain kidney and lungs were significantly affected (P<0.05) by the ingestion of low-protein diet while the activity of this enzyme in the liver is not significantly affected. In the brain, it is observed that the ingestion of low-protein diet led to significant increase (P<0.05) in the activity of this enzyme while a significant reduction was observed in the kidney and lungs when compared with the control.

Tissue superoxide dismutase activity is shown in Table 4. The activity of superoxide dismutase was significantly increased in the brain in the group of animals fed low-protein diet, significant reduction (P<0.05) was observed in the kidney while the liver and lung enzymes did not exhibit any significant change when compared with the control.

Discussion

The significant increase (P< 0.05) in the concentration of malondialdehyde observed in the tissues of animals fed low protein diet is an indication of increased lipid peroxidation in those tissues. Lipid peroxidation is assessed by maximal rate of malondialdehyde formation (Gaudin, 1991; Chield *et al.*, 1999). The result agrees with that of Tatli *et al.* (2000) who also reported increased malondialdehyde concentration in the serum of marasmic children when compared with the controls. The deposition of malondialdehyde in these tissues has various significance. In the brain for instance, elevated malondialdehyde levels it has been proposed to be one of the major mechanisms of secondary damage in traumatic brain injury (Weighand *et al.*, 1999). MDA levels in liver may also be used to investigate the oxidative damage of protein and lipoproteins which is a possible pathogenic mechanism for liver injury (Kojic *et al.*, 1998).

Reductions in the activity of superoxide dismutase and

Table 2: Mean value of malondialdehyde in the different tissues

| Tissue | Malondiadehyde Level in the tissue of rats fed | |
|--------|--|--------------------------------|
| | Adequate protein | Low protein diet |
| Brain | 2.82x 10 ⁻⁵ ± 0.20 | 4.38 x 10 ⁻⁵ ± 0.51 |
| Liver | 1.70 x 10 ⁻⁵ ± 0.68 | 3.25 x 10 ⁻⁵ ± 0.20 |
| Kidney | 0.27 ± 0.015 | 0.47 x 10 ⁻⁵ ± 0.01 |
| Lungs | 1.27 x 10 ⁻⁸ ± 0.02 | 2.00 x 10 ⁻⁸ ± 0.29 |

Results are mean of 4 determinations ± SEM.

Table 3: Catalase activities of tissues of rats maintained on adequate and low-protein diets

| Tissue | Specific activity in the tissue of rats fed | |
|--------|---|---------------------------------|
| | Adequate protein diet | Low protein diet |
| Brain | 0.55 ± 0.05 | 0.985 ± 0.035 |
| Liver | 0.400 ± 0.3 | 0.55 ± 0.45 |
| Kidney | 1.78 ± 0.01 | 1.72 ± 0.02 |
| Lungs | 3.3 x 10 ⁻³ ± 0.00032 | 1.5 x 10 ⁻³ ± 0.0011 |

Results are mean of 4 determinations ± SEM.

Table 4: Superoxide dismutase of tissues of rats maintained on adequate and low-protein diets

| Tissue | Specific activity of tissues of rats maintained on adequate and low-protein diets | |
|--------|---|------------------|
| | Adequate protein diet | Low protein diet |
| Brain | 1.87 ± 0.03 | 1.94 ± 0.005 |
| Liver | 1.89 ± 0.02 | 1.98 ± 0.008 |
| Kidney | 1.67 ± 0.10 | 1.44 ± 0.08 |
| Lungs | 1.84 ± 0.15 | 1.82 ± 0.11 |

Results are mean of 4 determinations ± SEM.

catalase as observed in the kidney and lungs may in part be due to reduction in the rate of the synthesis of these enzyme. The concentration of some plasma enzymes have been reported to be reduced in protein energy malnutrition reflecting depletion of these enzymes in the tissue (Kumari *et al.*, 1993).

The disorder in the supply of amino acid that occurs in protein energy malnutrition could also result in reduced synthesis of these enzymes (Holt *et al.*, 1963; Saunders *et al.*, 1967). In addition, Mates *et al.* (1999) reported that when oxidative stress arises as a consequence of a pathologic event, a defense system promotes the regulation and expression of antioxidant enzymes but in protein energy malnutrition, the defense system is weak to promote the regulation of antioxidant enzyme.

The significant reduction in the activity in tissue studied show that low – protein diet affects the activity of these enzymes. The implication of this finding is that there could be accumulation of free radicals in these tissues when there is inadequate protein levels in diet and free radicals are known to have deleterious effects on the biological system.

The activity of superoxide dismutase and catalase, the

enzymes whose natural interaction synergy constitute the most effective system of free radical control in the body (Gilbert, 1981) were significantly increased in the brain of the test animals when compared with the control group. The increase might be due to the fact that in response to increase in the concentration of free radicals, the biological system responds to this by increasing the activity of these antioxidant enzymes in order to be able to combat the increasing free radicals and put it under control (Tisan, 1995).

The activity of the enzymes was not significantly changed in the liver. It has been observed that despite the marked structural change reported in the liver during protein energy malnutrition, liver function is well maintained and severe liver failure is unusual.

This might imply that the liver is relatively spared probably because of its importance in general metabolism and detoxification of foreign compounds (Akanji and Onyekwelu, 1985, Talwar *et al.*, 1989).

The antioxidant defence system was affected in rats fed low protein diet. Reduced antioxidant status and increased oxidative stress occurs in the tissue in protein energy malnutrition. The alteration in the activity of the enzymes as well as enhanced lipid peroxidation could be early markers of protein energy malnutrition.

References

- Adelusi, S.A. and J.O. Olowokere, 1985. A rapid method of induction of post-natal protein-energy malnutrition (PEM) in laboratory animals. *Nig. J. Appl. Sci.*, 3: 171-174.
- Akanji, M.A., 1986. A comparative biochemical study of the interaction of some tyranosides with rats tissue cellular system. Ph D Thesis. University of Ife, Ile-Ife.
- Akanji, M.A. and M.A. Onyekwelu, 1985. Effect of repeated administration of suramin on alkaline phosphatase activity in rat kidney. *IRCS Med. Sci.*, 13: 710.
- Ashour, M.N., S.I. Salem and H.M. El-Gadban, 1999. Antioxidant status in children with protein energy malnutrition (PEM) living in Cairo. *Eur. J. Clin. Nutr.*, 53: 669-673.
- Ashru, K. and Sinha, 1971. Determination of catalase activity in white blood. *Advance in Enzymology and related areas of Molecular Biology*, 27: 380.
- Berkow and Robert, 1999. *The Merck Manual of Medical Information: Home edition*, White House Station. NJ Merck Research, pp: 104-108.
- Chield, R., S. Brown and S. Day, 1999. Changes in indices of antioxidant status, lipid peroxidation and inflammation in human skeletal muscle after eccentric muscle action. *Clin. Sci. Calch.*, 96: 106-115.
- Gaudin, A.J., 1991. Cardiovascular system anatomy. In: *Encyclopedia of human biology*. Vol. 1. Renato Dulbeccoo Ed. Academic Press Inc. N.Y.

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- Gilbert, D.L., 1981. Oxygen and living processes, and interdisciplinary approach. Springer N.Y.
- Holt, C., J. von Chang, M. Holt von and Bihm, 1963. BBA; 90:611.
- Holt, C.J., M. von Chang, Holt von and Bihm, 1963. Disorders in supply of aminoacids in protein energy malnutrition BBA, 90: 611.
- Ibukun-Olu Alade, 2001. Public Health Nutrition. 2nd ed. Tosco Press, Nigeria, pp:107-112.
- Kojic, G., P. Vlahonic and D. Ravloure, 1998. The possible importance of the cation binding site for the oxidative modification of liver nucleolidase. Arch. Physiol. Biochem., 106: 91-99.
- Kratzer, F.H., J.B. Allied, B.N. Davis, B.J.V. Marshall and P. Vohra, 1959. The effect of Autoclaving Soybean Protein and the addition of Ethylenediamine Tetraacetic acid on the biological availability of dietary Zinc for Turkey Poults. J. Nutr., 68: 313.
- Kumari, R., Y.N. Rao and B. Tralukdar, S. Agarwal and R.K. Puri, 1993. Serum Enzyme abnormalities in Protein Energy Malnutrition. Ind. Pediatr., 30: 469-473.
- Mates J.M., C. Perez-Gomez and Nune-z-de Castrol, 1999. Antioxidant enzymes and human diseases. Clin. Biochem., 8: 595-603.
- Misra, and Fridovich, 1972. Determination of the level of superoxide dismutase in whole blood. Yale Univ. Press New Haven, pp: 101-109.
- Plummer, D.T., 1974. An introduction to Practical Biochemistry. 2nd ed. McGraw-Hill. London, pp: 144.
- Saunders, S.J., G.O. Barbezat and W. Withman, 1967. Am. J. Clin. Nutri., 20: 760.
- Saunders, S.J., G.O. Barbezat, W. Withman and J.D.L. Hansen, 1967. Aminoaciduria of Kwashiokor. Am. J. Clin. Nutr., 20: 760.
- Sive, A.A., E.F. Subotzky and H. Malan, 1993. Red blood cell antioxidant enzyme concentration in kwashiorkor and marasmus. Ann. Trop. Paediat., 13: 33-38.
- Talwar, G.P., L.M. Srivastave and K.P. Mudgil, 1989. Textbook of Biochemistry and Human Biology. 2nd Ed Prentice Hall of India. Private Ltd.
- Tatli, M.M., H. Viral and A. Koc, 2000. Altered antioxidant status and increased lipid peroxidation in marasmic children. Pediat. Int., 42: 289-292.
- Tisan, L., A. Cai and R. Bowen, 1995. Effects of caloric restriction on age related oxidative modification of macro molecules and lymphocyte proliferation in rat. Free Rad. Biol. Med., 19: 859-865.
- Weighand, M.A., A. Laipple and K. Plascke, 1999. Concentration changes of malondialdehyde across the cerebral vascular bed and shedding of L-selectin during carolide endarterectomy. Stroke, 30: 306-311.
- Wills, D.E., 1985. Evaluation of lipid peroxidation in lipids and Biological membranes. In: Biochemical Toxicology: A practical approach. Ed. By K. Snell and B. Mullak. IRL. Press, Oxford.